Base-resolution Quantitative Sequencing Methods to Study RNA Modifications in Gene Expression Regulation

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The reversible N6-methyladenosine (m6A) methylation regulates messenger RNA (mRNA) fate and metabolism in various biological processes. Functional characterization of other mRNA modifications, such as pseudouridine (Ψ), 2'-O-methylation (Nm), 5-methylcytidine (m5C), and internal N7-methylguanosine (m7G), has been hampered by the lack of the sensitive and quantitative methods that can map these RNA modifications transcriptome-wide. In this talk, based on chemical approaches and enzyme engineering, I will introduce the quantitative sequencing tools to uncover multiple mRNA modifications at base resolution, and to monitor their modification fraction change through misincorporation and deletion signatures, including BID-seq, Nm-Mut-seq, m7G-quant-seq, etc. These methods accurately assigned the specific 'writer' protein for the modified sites and facilitated the discovery of 'reader' proteins that reveal the functional roles of these RNA modifications. Besides the RNA modifications on steady-state RNA, I will introduce DAMM-seq to site-specifically detect and quantify multiple RNA methylations simultaneously within mitochondrial nascent RNA, which demonstrated ALKBH7-mediated reversible RNA methylation to regulate the processing and structural dynamics of polycistronic mitochondrial RNAs. These new technologies set the stage to investigate the roles and mechanisms of multiple RNA modifications in gene expression regulation and diverse biological processes.